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Effects of Melatonin on *Clarias Macrocephalus* Female Broodstock Performance

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Abstract

This study examines the effects of exogenous melatonin treatment to the first sexual maturity stage in female broodstock of the *Clarias macrocephalus*. The gradual melatonin levels of Control (0 mg/kg melatonin), Mt0.05 (50 mg/kg melatonin) and Mt0.25 (250 mg/kg melatonin) in the diet mixed in isonitrogenous and isocaloric of 37% crude protein and 9.3% crude lipid was applied to the first sexual maturation female catfish. Estradiol analysis, maturation analysis, breeding performance and the larval quality were evaluated. The exogenous melatonin treatment increased the fecundity, gonadosomatic index and egg diameter ($p < 0.05$) after eight weeks of treatment. During artificial spawning, the melatonin enhanced the egg production and the larval survival rate ($p < 0.05$). The current results supported the concept of exogenous melatonin treatment may enhance the first sexual stage of maturation in the *C. macrocephalus*.

Keywords: Catfish; female; maturation; melatonin.

1. Introduction

The walking catfish (*Clarias macrocephalus*) is important aquaculture species in Thailand and Southeast Asia because of its tender flesh and acceptable flavor, high market value and big aquaculture potential.

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However, it is now regarded as near threatened by The International Union for Conservation of Nature (IUCN) Red List [1] and the lack of information of basic knowledge on their reproductive physiology, slow growth rate and disease susceptibility of *C. macrocephalus* has caused the condition to become more serious.

Photoperiod is one of the important factors for synchronizing the sexual maturation and reproduction in fish [2]. However, photoperiod manipulations vary depending on the species [3; 4; 5]. Melatonin is a hormone produced during the night by the pineal gland and the retina, and it is the hormone associated with the photoperiod. Melatonin signal is conveyed to the brain and other peripheral tissues such as ovaries, and it affects the circadian rhythms of animals [6; 7]. They also suggested that melatonin is involved in reproductive processes of the hypothalamus-pituitary-gonadal endocrine axis. Melatonin reaches the pituitary directly through pituitary melatonin receptors where it induces gonadotropin-releasing hormones (GnRHs) [8]. In vitro melatonin study on the Atlantic croaker with fully developed gonads, low concentrations of melatonin showed in vitro luteinizing hormone (GTH-II) released from pituitary cells in culture. The follicle stimulating hormone (GTH-I) is involved in the initiation of gametogenesis and regulation of gonadal growth, whereas GTH-II mainly regulates gonadal maturation, spermiation and ovulation [9; 10].

Attempts to enhance the gonadal maturation by inducing melatonin has been made in some fish species such as salmon, carps and zebrafish [11; 12; 13]. The aim of this research is to investigate the effects of exogenous melatonin administration to the first sexual maturity stage in female broodstock of the *C. macrocephalus*.

2. Materials and Methods

2.1 Animals

The maiden or first spawning *Clarias macrocephalus* female broodstock were obtained from the Fisheries Station of Kham Pheng Phet, Department of Fisheries, Ministry of Agriculture and Cooperative, Thailand, and the trial experiments were done in the Laboratory of Nutrition and Aquafeed, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand. The eighteen weeks old catfish were acclimatized in 500L tanks at the density of 15ind/m2/fish/tank and fed with control feed for two weeks prior to the experiment. The source of water supply for the experiment was aerated to maintain the oxygen supply in the experiment tank.

2.2 Experimental diets

Feeding trial used one control and two different levels of melatonin. The basal diet was formulated from practical ingredients containing 22% fishmeal, 35% soybean, 1% spirulina, 12% wheat flour, 11.8% tapioca, 5% ricebran, 2% fish oil, 3% soy oil, 1.2% mineral premix, 2% soy lecithin, 1.5% calcium phosphate, 1% attractant, 2% binder and 0.5% vitamin premix. The diet also consisted of 37% crude protein and 9.3% crude lipid. Diets containing melatonin were prepared by adding graded levels of melatonin (Health Connection Labs Inc, USA) to the basal diet. These melatonin concentrations were 0 (control), 50(Mt0.05) and 250 (Mt0.25) mg/kg in the diet [modified 14].

2.3 Experimental condition

A total of 45 female of mean weight 63.85 ± 4.97 g (mean ± S.D.) were starved in tanks for two days prior to the experiment and all experiment population were subjected to normal photoperiod (12 hours daylight) prior to treatment. The fishes were fed at a level equivalent to 3% of their body weight, and this amount of diet was divided into two equal feedings per day. The fishes were randomly distributed in three treatments (0, 50 and 250 mg/kg of melatonin) with three replicates. Following acclimation, the fishes were exposed to melatonin treatments for eight weeks. The catfish were six months old at the end of the experiment.

2.4 Growth performance

Female broodstock was weighted before the final sampling to determine the growth performance by using the following formula:

Weight gain (%) = [(Final body weight – Initial body weight) / Initial body weight] X 100

2.5 Estradiol analysis

The serum from treatments was used for estradiol analysis. The procedure followed the IMMULITE® Estradiol by Siemens Medical Solution Diagnostic. The estradiol enzymes conjugate competes with serum samples, and the chemiluminescent substrate was added and the signal was generated according to the bound enzyme.

2.6 Gonadosomatic index

The Gonadosomatic Index was determined [15] as:

GSI = 100 (Gm/Tm)

Where;

Gm = Mass of Gonad, Tm = Total mass of fish

2.7 Artificial breeding

After eight weeks of treatment, artificial breeding was done with the remaining female *C. macrocephalus* from each treatment to assess the reproductive performance. The fishes were taken from the treatment tanks and matured catfish were identified. Prior to breeding assessment, the broodstock were anaesthetized by using clove oil. The fishes were individually inspected to observe the external appearance of the fishes. Matured fish indication such as papilla colouring, abdominal swelling and swollen papilla were observed. The female *C. macrocephalus* were weighted prior to breeding session. A mixture of 30µg buserelin acetate (LHRH analogue) and 10mg domperidone (dopamine analogue) were induced to one kilogram female broodstock (0.1ml mixture for 100g females). The stripping was done after 16 hours of the artificial induction. During stripping, a gentle massage was done on the belly to release the eggs. Male African catfish semen was used as the control male for

the artificial spawning in order to standardize the male semen. The semen was diluted in normal saline water and separated for every replicates. The eggs and semen were added to the same bowl for fertilization, and the sperm were mixed with the eggs by stirring gently with the soft end of a brush. The fertilized eggs were then transferred to the hatching tanks for incubation.

2.8 Fish reproductive characteristics

After breeding session, egg production, egg quality and larval quality were determined. Egg production was estimated by direct counting of spawnable eggs in the female ovaries [16]. Thirty eggs from each sample were measured by using Motic microscope (Motic BA210 Digital Laboratory Microscope with Moticam 1000 camera) for egg diameter measurement. The total number of eggs and larvae were calculated. Fertilization rate, hatching rate and survival rate of the *C. macrocephalus* larvae were done by following the method of [17].

2.9 Statistical analysis

All data were analyzed by one-way ANOVA (analysis of variance), followed by the Tukey's honest significance test to analyze the significant between the treatment means where all means comparisons significance were tested at $P < 0.05$ by using SPSS software [18].

3. Results

Melatonin trial did not significantly affect the weight gain between treatments where the weight gain was 10.06 % (control), 11.73 % (Mt0.05) and 12.38 % (Mt0.25) with the value at $p = 0.6$. Similar result was found in estradiol profile of melatonin treated broodstock (mean ranging from 486.7– 2772.3pg/ml; $p = 0.2$) where the estradiol profile was not significantly different between treatments (Table 1). After the experiment trial, there was a significant increase in the gonadosomatic index (GSI) (Figure 1A), fecundity (Figure 1B), and egg diameter (Figure 1C) among female fish in the presence of melatonin after eight weeks of treatment with the mean ranging from 5.61 – 9.77%; $p = 0.001$, 3034.1 – 4838.0 eggs/g; $p = 0.003$, and 1448.4– 1555.1µm; $p = 0.001$, respectively (Table 1).

Table 1: Maturation analysis of female *C. macrocephalus* with different levels of melatonin

Treatment	Control	Mt0.05	Mt0.25	P value
Weight gain (%)	10.06 ± 6.0	11.73 ± 8.1	12.38 ± 4.9	0.6
Estradiol (pg/ml)	486	2478	2772	0.2
GSI (%)	5.61^{c}	7.77^{b}	9.77^{a}	0.001
Fecundity (egg/g)	3034.1^{b}	4203.1^{a}	4838.0^{a}	0.003
Diameter (µm)	1448.4c	1521.4^{b}	1555.1^{a}	0.001

$^{a, b, c}$ Values with different superscripts in a row differ significantly ($P < 0.05$).

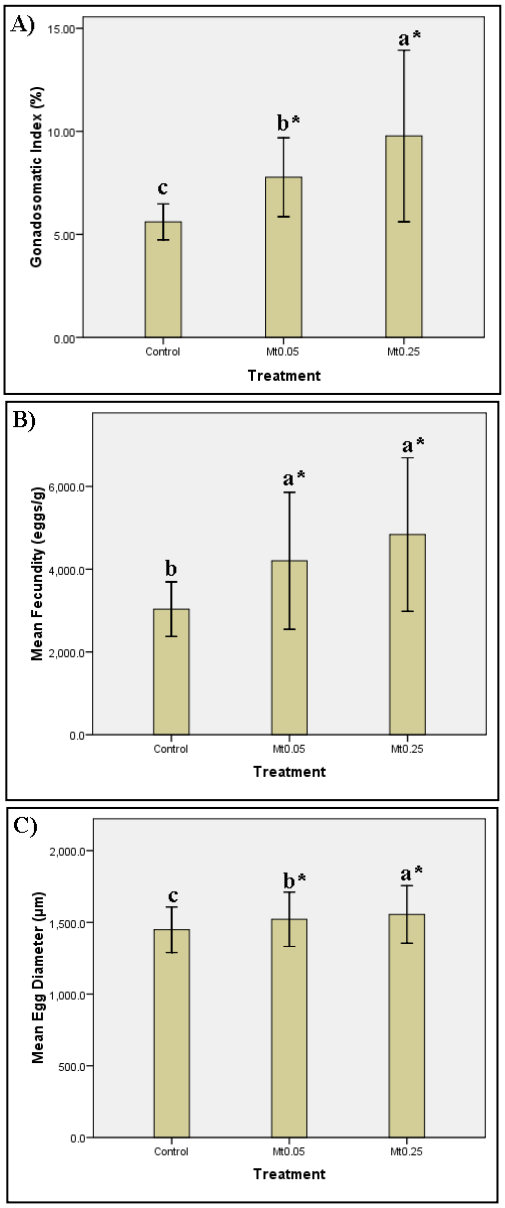

Figure 1: Mean gonadosomatic index (A), fecundity (B) and egg diameter (C) of C. macrocephalus after eight weeks of melatonin treatment. Values are expressed as mean ± SEM (GSI N: 6 animals/replicate, fecundity N: 6 animals/replicate, egg diameter N: 180 eggs/replicate). $p < 0.05$

After eight weeks of experiment trial, the female broodstock was artificially spawned with semen from control males to evaluate the reproductive performance. The egg production (Figure 2A) of the melatonin treated spawned female Mt0.05 and Mt0.25 were significantly higher (mean ranging from2368.7– 4065.9 eggs/g; p= 0.004) compared to the control groups (Table 2). There was higher number of larval survival rate (Figure 2B) (Table 2) observed in females broodstock exposed in melatonin treatment for C. macrocephalus to both doses of melatonin (mean ranging from 29.1 % – 77.1%; p=0.001). However, the melatonin treatment has no significant different in the fertilization rate (mean ranging from 65.75 % – 75.16%; p = 0.8) and hatching rate (mean ranging from 10.5 % – 20.3 %; p= 0.6).

Table 2: Maturation analysis of female *C. macrocephalus* with different levels of melatonin

Treatment	Control	Mt0.05	Mt0.25	P value
Egg production (egg/g)	2368.7b ± 732	4065.9a ± 735	3725.4a ± 1094	0.004
Survival rate (%)	29.1b ± 17.9	77.1a ± 1.4	67.8a ± 10.6	0.001
Fertilization rate (%)	65.75	75.16	67.37	0.8
Hatching rate (%)	10.5	20.3	16.8	0.6

[a, b, c] Values with different superscripts in a row differ significantly ($P < 0.05$).

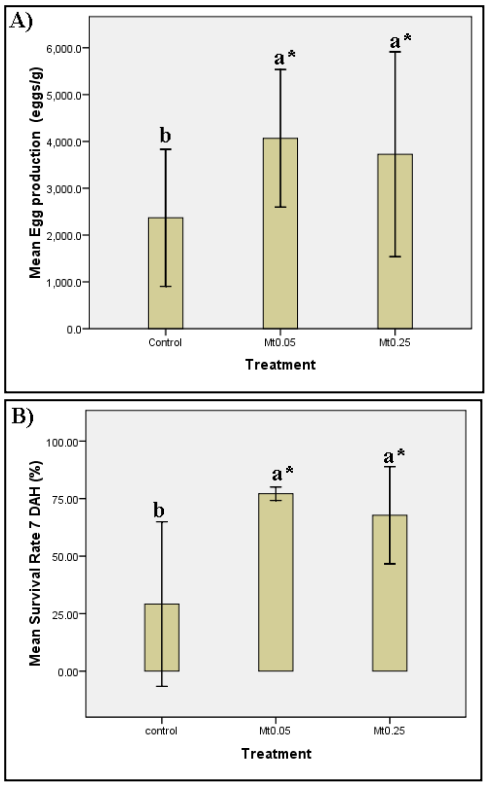

Figure 2: Mean egg production (A) and survival rate (B) after eight weeks of melatonin treatment. Values are expressed as mean ± SEM (n = 8). $p < 0.05$

4. Discussion

The estradiol profile for the *C. macrocephalus* female broodstock was not significant with melatonin treated feed at both doses compared to the control group. Generally, estradiol level will significantly increase after melatonin administration in mammals such as sheep [19]. However, the previous study stated that the level had significantly increased only from week 10 to 13. The insignificant estradiol level in melatonin treated group might be due to the timing of blood sampling at the end of oocytes maturation where the female broodstock

already developed maturation characteristic. According to [20], the plasma levels of estradiol produced by the ovarian follicles, paralleled with vitellogenin where estradiol profile showed maximum concentrations at the pre-spawning period, reaches a peak before maturation. Then, estradiol levels fall rapidly just prior to final oocytes maturation [21].

The levels of gonadosomatic index (GSI) and fecundity from both melatonin groups exhibited an increased significant pattern during the experiment trial compared to the control group. Similar findings have been reported in rat, salmon, *Channa punctatus*, zebrafish and Japanese medaka [22; 11; 23; 24; 25]. In the present study, the oocytes in the ovary exposed to both doses of melatonin showed a significant increase in size and maturation level based on the egg diameter analysis. Estradiol stimulated the vitellogenin synthesis in the liver during vitellogenesis and transported via circulation to the ovary. The vitellogenin is the precursor to yolk proteins and the primary protein contributing to oocyte growth [26]. Vitellogenin is then accumulated for oocytes development and resulted in atremendous increase in the values of GSI and egg diameter during spawning phase [27]. Intra-ovarian melatonin during spawning in carp is five times higher than measured during post-spawning and the study emphasizes on the importance of ovarian hormones, vitellogenin accumulation in the oocytes and relationship with seasonal changes in carp ovarian melatonin [28].

The present study revealed that there was a significant increase in egg production and larval survival rate after treated female fish were paired with male fish. These results indicate that this might be due to the increase of broodstock maturation quality in terms of GSI, fecundity and egg diameter in the treated female with melatonin. This is the first report on the effects of exogenous melatonin in egg production and larval survival rate therefore, supporting the hypothesis of a more prominent maturation process in females treated with melatonin.

5. Conclusion

The current results strongly support the concept of melatonin as one of the important hormone in regulating the reproductive system for *C. macrocephalus*. In addition, the melatonin also able to enhance the first sexual stage of maturation and female broodstock performance in this fish species. This knowledge could be relevant for aquaculture purposes, such as increasing the quality of the reproductive system and the larval production.

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